Multimodal imaging shows fibrosis architecture and action potential dispersion are predictors of arrhythmic risk in spontaneous hypertensive rats

Supporting Arrhythmia and Alternans Data

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Data collection

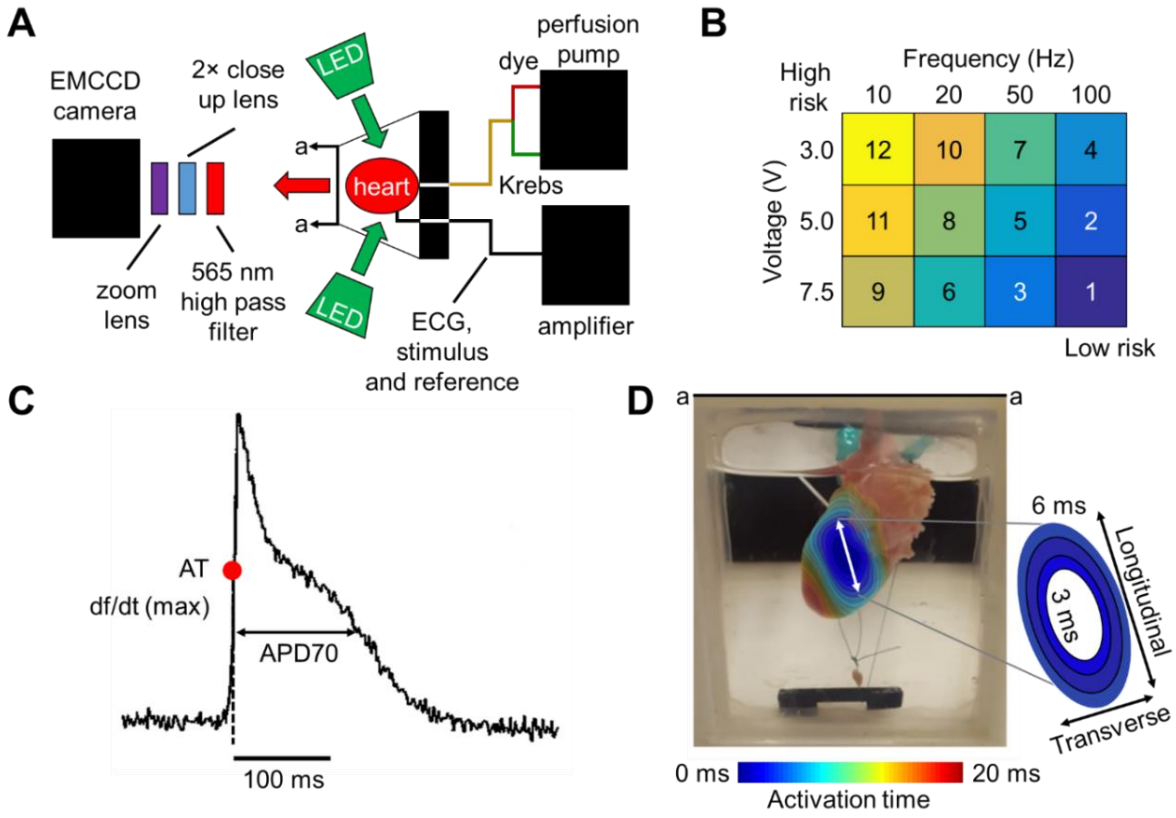

Figure 1. Experimental setup and methods. **A**. Schematic of experimental apparatus. **B**. A scale for VT/VF risk assessment constructed from the stimulus frequency and voltage at which arrhythmia occurred. **C**. Optical action potential with activation time (AT) and 70% action potential duration (APD70) indicated. **D**. Estimation of longitudinal and transverse conduction velocities from elliptical approximations to early AT maps between 3 and 6 ms. The viewpoint relative to the schematic in A is indicated by aa.

Optical mapping following left ventricular free wall pacing was conducted as shown in the schematic of Figure 1A. Arrhythmic risk was assessed using discrete stimulus amplitudes and burst frequencies over a 2 s period. The risk values associated with each amplitude and frequency combination are given in Figure 1B. Stimulus pulses were 2 ms duration. Action potential duration was measured at 70% of the return to baseline (Figure 1C) and conduction velocities were calculated by fitting ellipses to the activation time contours at 3, 4, 5 and 6 ms and finding the average of major (longitudinal) and minor (transverse) apex-to-apex distances across the three isochrone pairs (Figure 1D).

Conduction velocity

Raw conduction velocity data is given in the longitudinal direction in Table 1 and the transverse direction in Table 2. The anisotropy ratio is the longitudinal conduction velocity divided by the transverse velocity.

Table 1. Beat averaged (3 beats) longitudinal conduction velocity (m/s) at a range of pacing rates.

		Base Cycle Length (ms)									
Animal	Age (months)	429.2	413.2	335.6	282.5	243.9	214.1	191.2	172.7	156.3	142.9
1	12.2	0.68	0.63	0.64	0.65	0.71	0.65	0.56	0.56	0.54	
3	12.4	0.68		0.66		0.68	0.66	0.77	0.71	0.68	0.61
4	12.6	0.83	0.81	0.79	0.76	0.75	0.68	0.67	0.65		
5	12.6	0.88	0.86	0.86	0.80	0.79	0.76	0.73	0.71	0.62	0.64
6	12.8	0.56	0.58	0.60	0.55	0.57	0.54	0.52	0.50		0.52
8	5.9	0.91		1.01	0.93	0.92		0.89	0.97	0.97	0.95
9	6.5	0.88	0.77	0.70	0.71	0.70	0.72	0.68	0.65	0.54	0.53
10	6.5	0.83		0.80	0.79	0.78		0.73	0.71	0.68	0.65
11	6.7	0.76		0.80	0.76	0.72		0.65	0.58	0.55	0.54
12	6.7	1.01	1.02	1.05	1.04	0.98	0.93	0.93	1.00	0.97	0.97
13	18.0	0.49	0.60	0.54	0.50	0.53		0.66	0.62	0.76	0.66
14	18.0	0.62	0.63	0.62	0.62	0.58	0.56	0.53	0.55	0.53	0.48
15	18.2	0.68		0.81	0.72	0.68		0.65	0.49	0.45	0.45
16	18.3	0.99		0.85	0.83	0.78	0.65	0.63	0.55	0.66	0.58
17	18.4	0.68	0.69	0.71	0.69	0.67	0.65	0.62	0.59	0.51	0.41

Table 2. Beat averaged (3 beats) transverse conduction velocity (m/s) at a range of pacing rates.

		Base Cycle Length (ms)									
Animal	Age (months)	429.2	413.2	335.6	282.5	243.9	214.1	191.2	172.7	156.3	142.9
1	12.2	0.37	0.43	0.40	0.40	0.33	0.33	0.36	0.31	0.27	
3	12.4	0.37		0.38		0.41	0.32	0.33	0.33	0.30	0.28
4	12.6	0.40	0.39	0.34	0.32	0.32	0.29	0.30	0.26		
5	12.6	0.37	0.35	0.30	0.30	0.29	0.28	0.27	0.28	0.29	0.25
6	12.8	0.31	0.28	0.22	0.24	0.25	0.23	0.21	0.22		0.21
8	5.9	0.42		0.42	0.39	0.39		0.39	0.41	0.40	0.38
9	6.5	0.55	0.46	0.48	0.45	0.44	0.40	0.41	0.41	0.38	0.38
10	6.5	0.43		0.43	0.42	0.42		0.40	0.39	0.38	0.37
11	6.7	0.41		0.42	0.41	0.41		0.35	0.37	0.35	0.33
12	6.7	0.57	0.54	0.58	0.58	0.57	0.47	0.55	0.56	0.54	0.53
13	18.0	0.29	0.30	0.30	0.30	0.29		0.30	0.25	0.30	0.23
14	18.0	0.29	0.29	0.28	0.27	0.26	0.25	0.23	0.24	0.22	0.20
15	18.2	0.35		0.35	0.32	0.30		0.28	0.26	0.23	0.23
16	18.3	0.39		0.36	0.35	0.32	0.27	0.27	0.21	0.23	0.19
17	18.4	0.31	0.30	0.31	0.31	0.30	0.29	0.29	0.28	0.29	0.24

Action potential duration

Raw action potential data is given in Table 3 and the action potential duration dispersion in Table 4.

Table 3. Action potential duration (ms) averaged over pixels in the field of view.

		Base Cycle Length (ms)									
Animal	Age (months)	429.2	413.2	335.6	282.5	243.9	214.1	191.2	172.7	156.3	142.9
1	12.2	117.21	116.72	111.25	112.60	109.53	102.15	104.54	95.17		
3	12.4	93.82		104.63	102.23	95.55	91.65	87.55	88.92	84.77	75.57
4	12.6	118.45	123.90	125.22	123.31	107.94		103.44	99.94	92.69	
5	12.6	125.41	126.61	114.81	116.20	110.68	111.14	106.26	96.49	91.23	85.14
6	12.8	138.51	127.16	114.36	114.45	112.60	109.45	103.09	98.46	96.31	89.62
8	5.9	97.75		114.02	108.05	97.91	92.56	87.69	84.42	77.50	83.08
9	6.5	125.35	129.47	115.40	111.13	109.67	107.77		96.36	92.67	
10	6.5	107.80	105.56	97.20	105.40	101.39		96.28	89.77	87.22	85.87
11	6.7	127.66	119.95	122.64	118.64	113.61	108.34	104.97	101.20	100.30	99.00
12	6.7	101.99	97.13	86.46	95.45	96.18	93.07	90.53	82.76	80.68	
13	18.0	140.72	141.32	142.84	137.60	124.60		118.60	103.95	97.16	95.64
14	18.0	139.48	140.93	130.34	128.23	122.54	116.10	111.28	105.27	99.41	
15	18.2	103.66		105.41	94.30	93.80	92.34	89.01	90.20	83.60	82.57
16	18.3	139.42	123.76	128.27	125.79	120.50	114.09	110.56	107.16	99.17	
17	18.4	121.86	124.95	117.19	113.09	113.84	114.02	104.74	97.67	93.79	88.91

Table 4. Action potential duration dispersion.

		Base Cycle Length (ms)									
Animal	Age (months)	429.2	413.2	335.6	282.5	243.9	214.1	191.2	172.7	156.3	142.9
1	12.2	0.43	0.52	0.33	0.37	0.62	0.33	0.51	0.28		
3	12.4	0.34		0.54	0.44	0.49	0.43	0.45	0.52	0.53	0.52
4	12.6	0.73	0.51	0.51	0.45	0.51		0.44	0.62	0.59	
5	12.6	0.15	0.37	0.46	0.29	0.37	0.77	0.63	0.72	0.62	0.73
6	12.8	0.31	0.27	0.24	0.47	0.27	0.30	0.30	0.33	0.37	0.37
8	5.9	0.44		0.47	0.39	0.36	0.20	0.16	0.10	0.28	0.12
9	6.5	0.14	0.32	0.25	0.18	0.32	0.29		0.41	0.52	
10	6.5	0.35	0.36	0.35	0.37	0.37		0.28	0.26	0.22	0.39
11	6.7	0.27	0.21	0.20	0.19	0.23	0.35	0.35	0.31	0.28	0.42
12	6.7	0.27	0.29	0.23	0.48	0.39	0.35	0.30	0.37	0.47	
13	18.0	0.39	0.46	0.41	0.29	0.56		0.57	0.55	0.63	0.71
14	18.0	0.46	0.42	0.56	0.46	0.42	0.34	0.41	0.44	0.58	
15	18.2	0.52		0.48	0.47	0.45	0.59	0.52	0.35	0.40	0.46
16	18.3	0.36	0.35	0.27	0.41	0.45	0.57	0.66	0.73	0.74	
17	18.4	0.37	0.25	0.24	0.36	0.25	0.47	0.41	0.32	0.43	0.66

Induction and duration of arrhythmia

Ventricular tachycardia or ventricular fibrillation (> 5 s duration) was induced in 17% of 6 month, 83% of 12 month and 100% of 18 month SHR cohorts. The median duration of arrhythmia induced in 6 month SHRs was 3.7 s, in 12 month 11.7 s and in 18 month 43.4 s. Figure 2 shows the distributions of arrhythmia durations. The longest 6 month SHR arrhythmia duration was 5.3 s. One 12 month SHR had a duration shorter than this (2.4 s) and all 18 month SHRs had arrhythmia durations longer than this, with the shortest being 6.7 s. The longest 12 month SHR arrhythmia duration was 17.3 s and the longest 18 month SHR arrhythmia duration was 94.9 s. The 18 month SHRs display a bimodal duration distribution; group 1: 6.7 s, 10.5 s and 12.9 s; group 2: 87.0 s and 94.9 s.

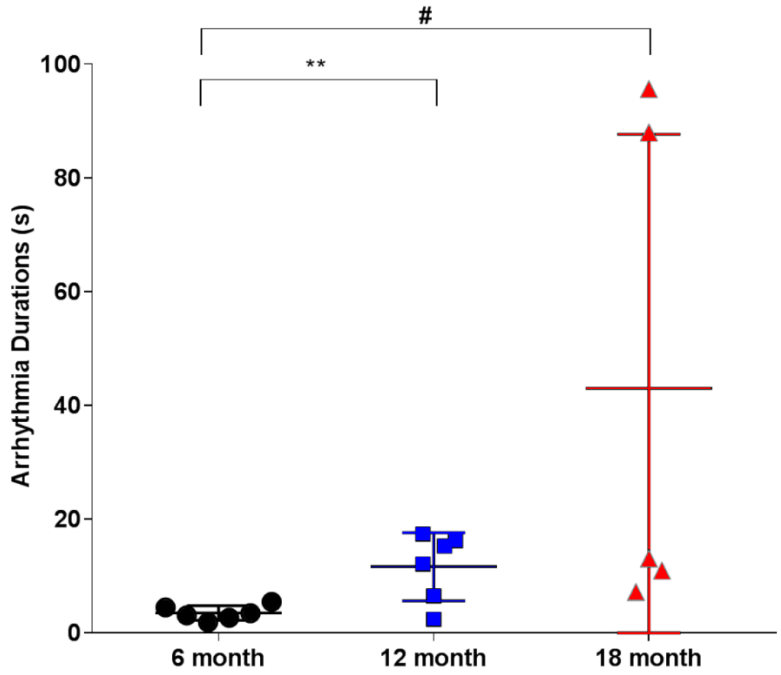

Figure 2. Duration of arrhythmia induced by burst pacing in each age cohort (6 month, n=6; 12 month, n=6; 18 month, n=5). Both 12 month SHR and 18 month SHR had longer duration of induced arrythmia compared to 6 month SHR (** $p<0.01$ and # $p<0.05$).

Rate dependent alternans

In all animals, 1:1 capture failed as the pacing rate increased and 2:1 block occurred. Over a range of base cycle lengths (BCL) prior to loss of 1:1 capture, alternans rhythm was observed in optical action potential amplitudes for all animals and activation times for most SHRs. Table 5 summarizes the BCL thresholds and the observed nature and prevalence of alternans. Figures 3 to 5 illustrate alternans rhythms in 6, 12 and 18 month SHRs, respectively. Overall, SHRs displaying discordant activation time (AT) alternans prior to loss of capture had significantly higher arrhythmic risk (8.1±4.1; $p < 0.005$) compared to those showing concordant or no AT alternans (3.9±4.1). SHRs with discordant AT alternans were associated with a significantly higher patchy fibrosis index (0.2±0.1) compared to those displaying concordant AT alternans (0.07±0.09; $p < 0.05$) and those displaying no AT alternans (0.02±0.02; $p < 0.05$).

Table 5. Summary of observed alternans onset conditions and categorization. BCL: base cycle length of pacing; AMP: amplitude; AT: activation time.

	6 month (n=6)	12 month (n=6)	18 month (n=5)
Conduction block (ms)	BCL < 129±10	BCL < 145±21	BCL < 136±14
Observed alternans (ms)	BCL < 139±9	BCL < 165±21	BCL < 161±14
Discordant AMP alternans (% animals)	100	100	100
No AT alternans (% animals)	50	0	0
Concordant AT alternans (% animals)	33	67	40
Discordant AT alternans (% animals)	17	33	60

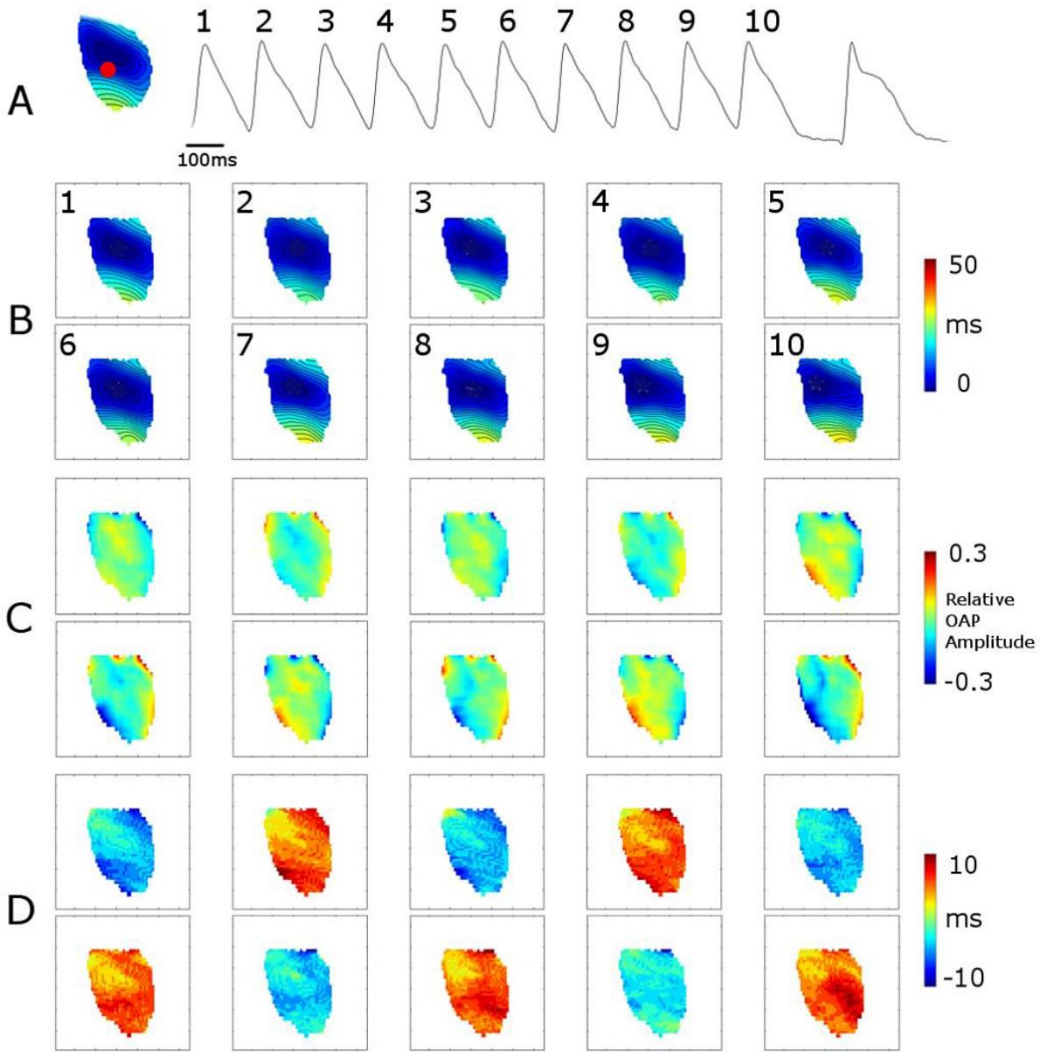

Figure 3. Activation and alternans rhythm observed in 6 month SHR over 10 beats prior to 2:1 block for BCL of 122 ms. **A.** Optical action potential trace at indicated site on the LV free wall. **B.** Activation time sequence for each of 10 beats. 2 ms isochrones. **C.** Optical action potential (OAP) discordant alternans shown by relative difference between current and previous beat. **D.** Activation time concordant alternans shown by difference between current and previous beat.

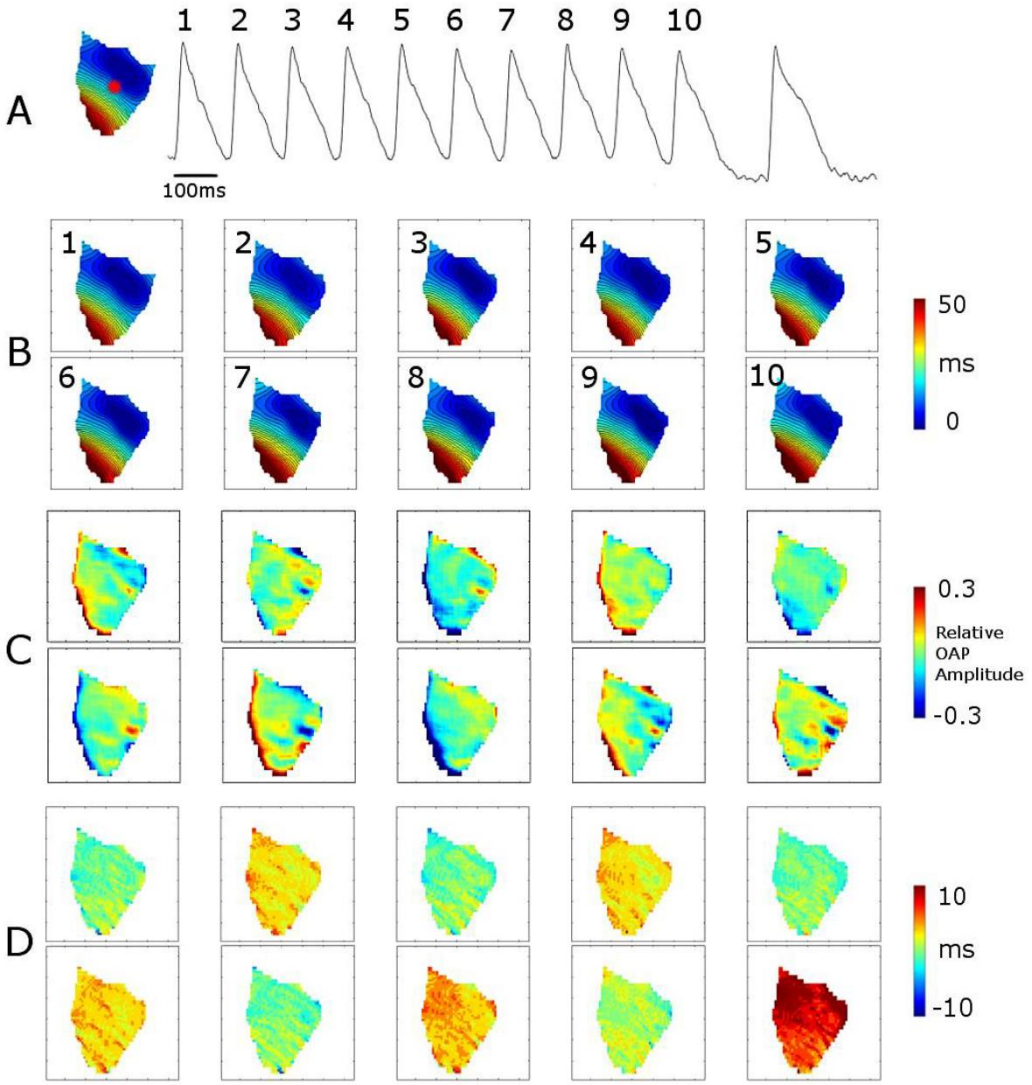

Figure 4. Activation and alternans rhythm observed in 12 month SHR over 10 beats prior to 2:1 block for BCL of 143 ms. **A.** Optical action potential trace at indicated site on the LV free wall. **B.** Activation time sequence for each of 10 beats. 2 ms isochrones. **C.** Optical action potential (OAP) discordant alternans shown by relative difference between current and previous beat. **D.** Activation time concordant alternans shown by difference between current and previous beat.

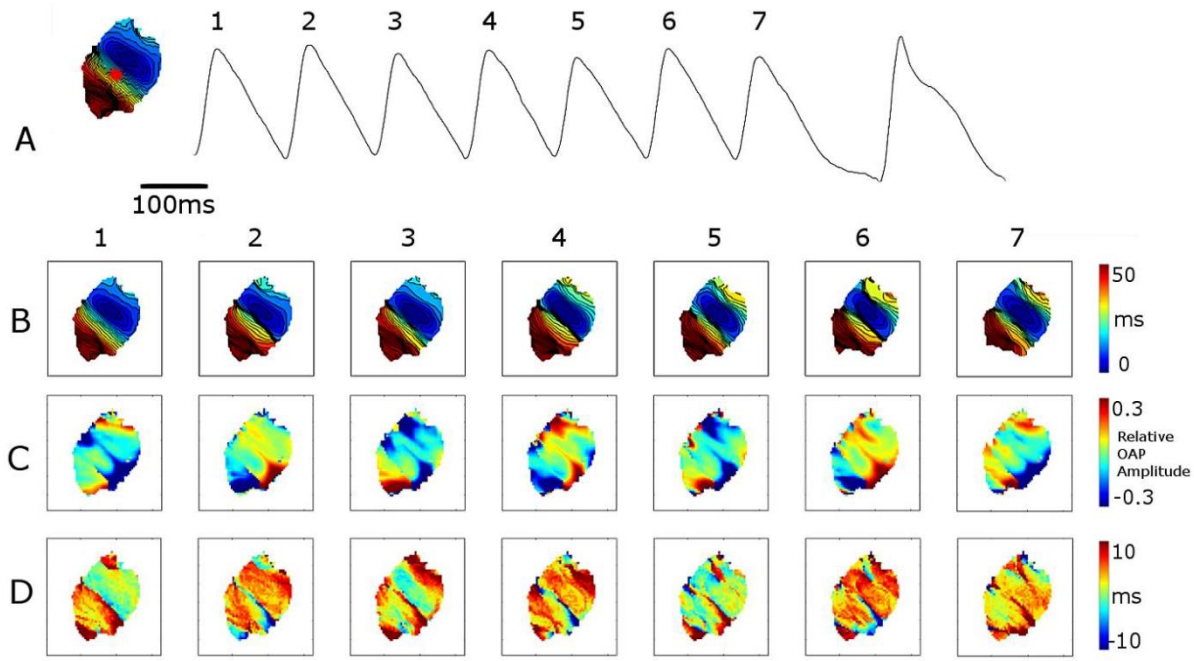

Figure 5. Activation and alternans rhythm observed in 18 month SHR over 7 beats prior to 2:1 block for BCL of 139 ms. **A.** Optical action potential trace at indicated site on the LV free wall. **B.** Activation time sequence for each of 7 beats. 2 ms isochrones. **C.** Optical action potential (OAP) discordant alternans shown by relative difference between current and previous beat. **D.** Activation time discordant alternans shown by difference between current and previous beat.